Removal of Invasive Honeysuckle (*Lonicera maackii*) and the Diversity and Abundance of Terrestrial Invertebrates

Thesis

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Abstract

The purpose of this study was to determine what effect, if any, the removal of honeysuckle (*Lonicera maacki*) has on the diversity and abundance of terrestrial invertebrates in riparian areas. The study took place at 10 different sites along the Little Miami River in Greene County, Ohio. There were 4 sites where honeysuckle had been successfully removed, 3 where the site was fully invaded, and 3 that had had removal done, but had experienced some comeback. Leaf litter samples were collected at each site a total of 3 times. After collection, the leaf litter was placed in Berlese funnels to extract the terrestrial invertebrates. Once the invertebrates had been extracted, they were counted, identified to the taxonomic level of Order, and then dried to determine biomass. Data analysis showed that there was no significant difference between the abundance, diversity or biomass of the invertebrates found between the different types of sites. Future research may want to explore a similar question but identify the invertebrates to the level of Species.

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Introduction

Amur honeysuckle (*Lonicera maackii*, hereafter referred to as honeysuckle), is an understory shrub that is native to east Asia and was first introduced to the United States for use as an ornamental plant in 1896 (USDA Forest Service, 2005). Honeysuckle is found in at least 24 states east of Mississippi River where it threatens native species and ecosystems (Hutchinson & Vankat, 1998). The shrub produces red berries that ripen in the fall and are often eaten by birds that disperse the seeds and have helped the honeysuckle expand its range (Hutchinson & Vankat, 1998).

As an invasive species, Amur honeysuckle has varied and wide-ranging effects on the ecosystems that it is found in. Honeysuckle is known to create a dense monoculture of vegetation, which can affect the richness, growth, production, and reproduction of many native plants (USDA Forest Service, 2005). Honeysuckle can be found in a variety of different conditions, including wet riparian areas, areas with full shade, and areas with full sun. This, along with its ability to tolerate harsh conditions (e.g. pollution, severe drought, and cold winter temperatures), help make it a successful invader (USDA Forest Service, 2005). According to a study by Hutchinson & Vankat (1998), honeysuckle is prevalent in forested areas, especially those that are young and/or have been disturbed. These researchers also found that honeysuckle is not well-established in cultivated areas, likely due to the lack of birds, who often serve as a dispersal mechanism for the seeds. It is suspected that honeysuckle has allelopathic effects on other plants and on herbivores (Cipollini et al., 2008). Allelopathic effects on other plants could help explain some of honeysuckle's success as an invasive shrub.

Due to its status as a widespread, invasive species, there is currently much literature on the effects of honeysuckle on different aspects of ecosystems. Specifically, there is a growing body of literature on the effects of honeysuckle on arthropods and other terrestrial invertebrates, but this literature is incomplete and often conflicting. Clear information in this area is necessary to make good management decisions because changes in the community structure of these organisms could have consequences for other aspects of the ecosystem through their interactions with the soil and their predators. This information can also be used to better understand the overall health of the ecosystems that the honeysuckle has invaded.

The objective of this study was to expand on this area of research by exploring the effect of the removal of honeysuckle from riparian areas on the diversity and abundance of the terrestrial invertebrates found in the surrounding environment. It was hypothesized that there would be a significant difference in the terrestrial invertebrate abundance and diversity between plots with differing honeysuckle removal statuses and that plots that had not undergone removal would have lower diversities of invertebrates than plots that had experienced removal. It was also hypothesized that invertebrate abundance would differ between plots depending on the specific taxonomic Order and that Order's characteristics (i.e. herbivorous shredders may be more prevalent at sites that are removed of honeysuckle due to their diet and the unpalatable chemical compounds that honeysuckle produces).

Literature Review

Changes in the diversity and density of plant cover from honeysuckle invasion could have consequences for fauna in the ecosystem. A study by Watling and others (2011), found that both the richness and evenness of amphibian species were lower in plots that were invaded by honeysuckle when compared with those that were not. The researchers attribute this effect to the density of the honeysuckle growth lowering the local temperature of the area (Watling et al., 2011). This study illustrates just how wide-ranging and not complex honeysuckle's effects can be because even though it was not directly interacting with the amphibians, its invasion still had consequences for them. Such interactions have the potential to alter food-web dynamics.

Avian fauna, in particular, may experience disturbances when invasive plant species, such as honeysuckle, are present in their habitats. One study found that American Robins (*Turdus migratorius*) that nested in invasive shrubs (*Lonicera maackii* and *Rhamnus cathartica*) experienced higher rates of nest predation than those that nested in native shrubs and trees. The researchers concluded that this was due to a combination of factors, including lower nest height and the absence of thorns that could deter predators on the invasive shrubs (Schmidt & Whelan, 2001).

Invasive plant species, such as honeysuckle, are often referred to as ecological traps for wildlife due to the fact that the wildlife may exhibit a preference for using the plants as habitat and/or food, but may be detrimentally impacted by this preference. A study by Rodewald and others (2009) examined the veracity of the use of this term by examining the effects of honeysuckle (*Lonicera maackii*) and the multiflora rose (*Rosa multiflora*) on nesting Northern Cardinals (Cardinalis cardinalis) across time. They concluded that invasive plant species may be more accurately referred to as "ephemeral ecological traps" due to their findings that the survival rates for young birds in nests built in honeysuckle were not consistently low throughout the season, despite starting out that way. In fact, nests built in the honeysuckle exhibited higher survival rates than those built in the native shrubs late in the season (Rodewald et al. 2009).

High densities of honeysuckle in a habitat may not always be detrimental to the species that live there. One study that looked at the behavior and development of gray catbirds (*Dumetella carolinensis*) in habitats with differing honeysuckle densities found that honeysuckle can have null and potentially positive effects when used as a nesting habitat. The researchers did not find significantly higher predation rates in the nests built in honeysuckle when compared to those built in native shrubs. The researchers did, however, find that the birds that nested in the honeysuckle made more trips to feed their young, spent less time during each trip, and that the young were of similar or better condition when compared with those raised in native shrubs (Gleditsch & Carlo, 2014). This demonstrates that the effects of invasive plants on native species are variable and often specific to the ecological context.

Many native insects have close ecological associations with native plants and so increasing abundances of non-native and invasive plant species, such as honeysuckle, could threaten them (Missouri Botanical Garden, n.d.). Non-native and invasive plant species may support lower diversities and abundances of arthropods when compared with native plant species. A study by Ballard and others (2013) looked at differences in leaf-feeding arthropod community composition on native and non-native early successional plants. The study found that the non-native plants supported lower diversities, biomasses, and abundances of arthropods than the native plants did (Ballard et al., 2013). Although honeysuckle was not one of the non-native species tested in the study, it is also known to be found in disturbed habitats, so it may similarly support lower diversities, biomasses, and abundances of arthropods. Species at higher trophic levels of the ecosystem could experience bottom-up controls from these changes.

In addition, the impacts of honeysuckle on insect species are not uniform. One study in Ohio showed that Hexapods were more abundant, diverse, and rich in plots with honeysuckle than in plots without (Loomis & Cameron, 2013). The researchers believe this finding was due to increased vegetative cover density because honeysuckle allocates more energy to stem and leaf growth than native shrubs, such as spicebush, under the same conditions. These findings differed from those of previous studies where increased honeysuckle density was not found to have had an effect on terrestrial invertebrate richness. One such study found that overall, arthropod abundance was not affected by the presence of honeysuckle, but that the abundance of Araneae was negatively impacted by the presence of honeysuckle and the abundance of Acari was positively impacted by the presence of honeysuckle. The researchers also found that arthropod abundance and diversity were not significantly different between plots where honeysuckle was absent and where honeysuckle had been removed, indicating that it does not take long (2 years) for arthropod communities to return to pre-honeysuckle conditions (Christopher & Cameron, 2012). Further studies would be needed to address and clarify honeysuckle’s interactions and effects on terrestrial invertebrates in varying habitats and circumstances. Yet, another study by Masters and others (2017) found that one year after removal, there was no difference in total abundance between plots that were invaded with honeysuckle and plots where it had been removed, but that there was a slightly higher diversity in the plots where honeysuckle had been removed. Three years after removal, the plots that were invaded with honeysuckle had higher diversity and overall arthropod abundance, but herbivore abundance was much higher in the plots where honeysuckle had been removed (Masters et al., 2017). A study by Mahon & Crist

(2019) found that earthworm density and biomass were not affected by honeysuckle by itself, but only by the interactive effect of honeysuckle and deer, where they found that honeysuckle removal combined with deer exclusion resulted in decreased earthworm biomass (Mahon & Crist, 2019). When looking at the same plots and treatments, but instead looking at their effects on ants, the researchers found that honeysuckle removal reduced the abundance and richness of the ants. The researchers attributed these findings to honeysuckle invasion creating favorable microclimates for the ants and providing them with increased vegetation structure (Mahon & Crist, 2019).

Certain species, such as pollinators and treehole mosquitoes, have been shown to be negatively affected by the presence of honeysuckle. One study showed that insect pollinator visits to a native Ohio herb, *Geranium maculatum*, were significantly reduced when honeysuckle was also present in the plot (McKinney & Goodell, 2010). This study concluded that the mechanism causing this change was increased shade in the understory. The researchers were able to rule out competition for pollinators because removing honeysuckle flowers did not increase pollinator visitation. Another study showed that oviposition by treehole mosquitoes (*Aedes triseriatus*) decreased with increasing honeysuckle density (Conley et al., 2011). This observed change in behavior of treehole mosquitoes in the presence of honeysuckle could have consequences for disease risk models, as treehole mosquitoes are vectors of the La Crosse virus.

In addition to affecting light availability and the density of other understory plants, honeysuckle is thought to have allelopathic effects. Cipollini and others (2008) showed that honeysuckle leaves contain phenolic compounds (including apigenin and chlorogenic acid), which can have inhibitory effects on other plants and insects. The researchers in this study found that extracts of honeysuckle leaves were able to strongly inhibit the growth of another plant

(*Arabidopsis thaliana*). It was also observed that an insect (*Spodoptera exigua*) exhibited a strong feeding preference for the control diet when given the choice between that and leaves treated with extracts from honeysuckle. This study noted that the concentration of allelopathic compounds in plants vary temporally and from plant to plant and so the inhibitory effects of the compounds can also vary (Cipollini et al. 2008).

The range of honeysuckle and other invasive shrubs has been shown to overlap with the range of the invasive gypsy moth (*Lymantria dispar*). McEwan and others (2008) found that gypsy moth caterpillars minimally consumed the leaves of honeysuckle and that when they did, they experienced adverse effects. This led the researchers to conclude that honeysuckle likely produces some chemical compounds that make it unpalatable for generalist insect herbivores. The researchers further noted that honeysuckle may benefit from the presence of this invasive moth species because the moth feeds on native shrubs, such as spicebush and pawpaw, due to the higher amount of nutrients that these species provide it with (McEwan et al., 2008). This preference for native shrubs may give honeysuckle an advantage in areas where these two invasive species coexist.

Other invasive shrub species have been shown to have effects on arthropod diversity and abundance. One study found that another invasive plant species, Japanese barberry, was associated with changes in arthropod community structure including decreased arthropod diversity and abundance (Clark & Seewagen, 2019). This study noted that although there were changes in diversity and abundance of arthropods in sites with Japanese barberry, the overall biomass did not differ significantly because the arthropods associated with Japanese barberry were significantly larger. Additionally, Clark and Seewagen (2019) found a significant decrease in predatory arthropods in plots that had been invaded by Japanese barberry. Another study looked at the effects of Chinese Privet (*Ligustrum sinense*) on butterfly species (Hanula & Horn, 2011). This study found that butterfly species were significantly more diverse and abundant at sites where invasive Chinese privet had been removed. The researchers noted that butterfly communities in other areas would likely benefit from the removal of other invasive species that tend to create dense monocultures (Hanula & Horn, 2011). As honeysuckle similarly can form dense monoculture thickets, these findings regarding Japanese barberry have implications for sites that are invaded with honeysuckle, suggesting that its removal could benefit butterfly communities.

Since it is a non-native species, honeysuckle's leaf-litter has characteristics that differ from those of native species. According to McNeish & McEwan (2016), honeysuckle leaf-litter has been shown to break down faster, have greater nitrogen content, and have lower lignin concentrations than certain native plant species (e.g. white ash, *Fraxinus Americana* L.). These differences also mean that it supports different soil microbial communities than native species (McNeish & McEwan, 2016). For example, when honeysuckle leaf extracts were added to live soil, orange jewelweed (*I. capensis*) experienced a lowered rate of mycorrhizal infection, which led to stunted growth because mycorrhiza is a fungus that aids in nutrient uptake (McNeish & McEwan, 2016). Extracts from the roots and shoots of *L. maacki* have also been shown to reduce the germination of some types of native plants (e.g. tall thimbleweed, *Anemone virginiana* L.) (McNeish and McEwan 2016). Since honeysuckle can affect the composition of soil microbial communities, it would not be unreasonable to assume that it may also affect the functioning of these communities and perhaps also the arthropods that aid soil processes through roles such as shredding and grazing. Understanding the diverse effects of Amur honeysuckle on native ecosystems in different ecological settings is necessary in order to develop and implement effective management strategies for dealing with this invasive species.

Invasive plants, such as honeysuckle, often co-occur with other invasive species, so it is important to understand the combined ecological effects of invasive species. The combined ecological effects of Chinese privet (*Ligustrum sinense*) and Amur honeysuckle (*Lonicera maackii*) were studied by Kuebbing et al. (2014). These researchers focused on forests near Knoxville, Tennessee and showed that the co-occurrence of the invasive species had an additive effect on the prevalence of subdominant invasive species and there were different soil pHs between the control and mixed plots. Based on the data collected, it was concluded that the combined effects of co-occurring invasive plant species do not have to be equal to the sum of their individual effects (Kuebbing et al. 2014). This implies the importance of studying the impacts of invasive species occurring together. The authors suggested further research in the area of co-occurring invasive species in order to develop better management practices.

Methods

<u>Study Location</u>

The study area was located in southwestern Ohio, in Greene County, along the Little Miami River. The Little Miami River watershed drains 1,758 square miles of land (Ohio Environmental Protection Agency, n.d.). The land cover of the eastern portion of the watershed is dominated by cultivated crops and pastureland, whereas the western portion is a mixture of multiple land uses that include forest, pastureland, and urban development (Ohio Environmental Protection Agency, n.d.). Honeysuckle is known to be a dominant invasive shrub in many forests in southwestern Ohio and is prevalent within the Little Miami River watershed (Hutchinson & Vankat, 1997).

Samples of leaf litter were collected at ten sites along the Little Miami River that had varying degrees of honeysuckle invasion and removal. Sampling sites were selected based on topography, relative proximity, honeysuckle prevalence, and contiguous land cover (for additional detail on site selection see DeJong, 2020). As part of a larger study, vegetation surveys were performed at each site to quantify the amount of honeysuckle and other plants present at each site. These surveys consisted of such actions as, visually estimating the percent of different types of ground cover within the site, measuring the Diameter at Breast Height (DBH) of all woody plants with heights above 3 m and DBH greater than 8 cm, among others (additional detail is provided in DeJong, 2020). The sites were located on both public and private lands and records detailing honeysuckle invasion and removal at these locations were provided by the landowners. The type of honeysuckle removal varied from site to site and included methods such as, herbicide treatment and physical cutting of the honeysuckle. The time since removal also varied from site to site, but all removal had been done at least 3 years prior to this study.

Terrestrial invertebrates found in the leaf letter samples from each site were identified and analyzed for abundance, mass, and diversity.

Figure 1: Map indicating the location of Greene County within Ohio.

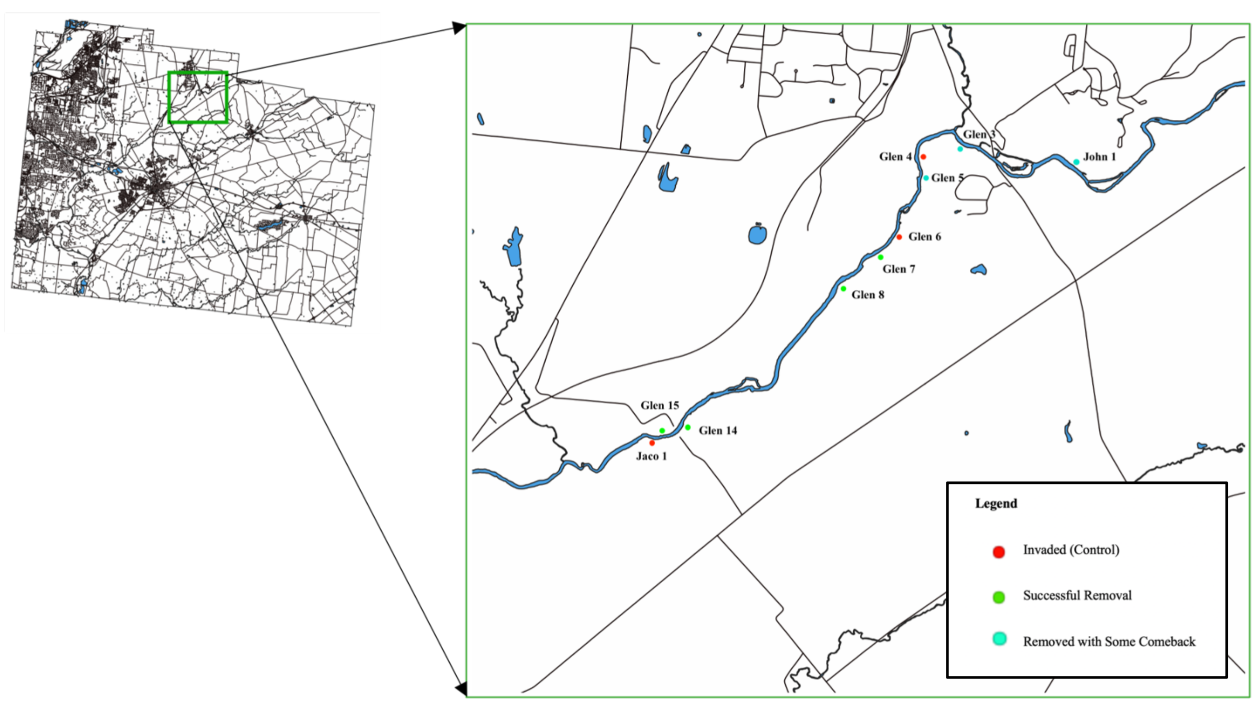

Figure 2: Map indicating the site locations along the Little Miami River.

Sample Collection

Leaf litter was collected at ten sites over the period spanning from May to early July, 2019. The purpose of this collection was to gather terrestrial invertebrates from the sites. Each site was visited between 10:30 AM and 4:00 PM on three separate occasions. At least two weeks separated the collection visits at each site.

Table 1. Sampling schedule for each site.

Site	Site Type	Sampling Round	Sampling Date
Glen 4	Invaded (Control)	1	5/20/19
Glen 4	Invaded (Control)	2	6/8/19
Glen 4	Invaded (Control)	3	7/5/19
Glen 6	Invaded (Control)	1	5/16/19
Glen 6	Invaded (Control)	2	6/8/19
Glen 6	Invaded (Control)	3	7/5/19
Jaco 1	Invaded (Control)	1	5/14/19
Jaco 1	Invaded (Control)	2	6/1/19
Jaco 1	Invaded (Control)	3	6/18/19
Glen 7	Successful Removal	1	5/16/19
Glen 7	Successful Removal	2	6/8/19
Glen 7	Successful Removal	3	7/5/19
Glen 8	Successful Removal	1	5/16/19
Glen 8	Successful Removal	2	6/8/19
Glen 8	Successful Removal	3	7/5/19
Glen 14	Successful Removal	1	5/14/19
Glen 14	Successful Removal	2	6/1/19
Glen 14	Successful Removal	3	6/18/19
Glen 15	Successful Removal	1	5/14/19
Glen 15	Successful Removal	2	6/1/19
Glen 15	Successful Removal	3	6/18/19
Glen 3	Partial Removal	1	5/14/19
Glen 3	Partial Removal	2	6/1/19
Glen 3	Partial Removal	3	6/18/19
Glen 5	Partial Removal	1	5/16/19
Glen 5	Partial Removal	2	6/8/19
Glen 5	Partial Removal	3	7/5/19
John 1	Partial Removal	1	5/14/19
John 1	Partial Removal	2	6/1/19
John 1	Partial Removal	3	6/18/19

The process for collecting the samples at the sites was as follows: 1) a cardinal direction was randomly chosen (without replacement) while standing in the center of the plot, 2) a 0.5 m^2 square frame was placed five meters from the center of the plot in the randomly chosen cardinal direction, and 3) the leaf litter inside the frame was gathered for the same amount of time at each collection site. The leaf litter that was collected was then stored inside a cloth pillowcase that was sealed with a rubber band. Leaf litter was stored in these pillowcases in the shade for up to four hours before being emptied into a Berlese Funnel. Berlese Funnels were used to extract the terrestrial invertebrates from the leaf litter samples by creating a desiccation gradient where the invertebrates in the leaf litter move away from a heat source and fall into a collection vessel (**Figure 3**). The collection vessel contained a 70% denatured ethanol solution to preserve the invertebrates. The heat source in the Berlese Funnels was a 40-Watt lightbulb that also dried the leaf litter. Each sample was hung in the Berlese Funnel for at least two days to ensure that it was completely dry. For most of the samples, the leaf litter was mixed or turned over during the drying period. Dry time was documented for each sample.

Before placing the leaf litter samples in the Berlese Funnels, the total wet mass was measured. Each sample was weighed again after its time in the Berlese Funnel to determine dry mass and calculate the change in mass. When drying was complete, the ethanol solution containing the invertebrates was transferred from the collection vessels of the Berlese Funnels to plastic bags that were sealed and labeled with sample identification information and stored at room temperature. Each ethanol sample was sorted through individually to separate the invertebrates from any soil and organic material that also passed through the Berlese funnel. The amount of soil in each sample was ranked on a scale of 1 to 3 with 1 being a small amount and 3 being a large amount. Once the invertebrates were separated from the soil, they were placed in vials labeled with the site name and sampling date that contained 70% denatured ethanol to preserve them. Every invertebrate that was collected was identified to its taxonomic order and then placed in a new vial labelled with the order name. The number of insects in each order was counted and recorded during the identification stage. Once all of the arthropod samples had been identified and counted, they were weighed, dried at 105°C for about 5 days, and then weighed again to get the dry weight of each sample. Dry weight was chosen as the biomass metric for the invertebrates due to the fact that they had been separated from almost all sediment and leaf material during the sorting and identification processes.

Figure 3: The Berlese Funnels that were used to dry the leaf litter samples and extract the insects.

Data Analysis

After identifying all of the collected samples to their taxonomic order, drying, and weighing them, the collected data was analyzed. First, the average counts of each order for each site were determined. When computing the average, the number of adults and other life-stages (e.g. larvae) were combined for each order. After determining the average counts, this information was used to calculate the Shannon Diversity Index value for each site. The Shannon Diversity Index is a mathematical measure of the species diversity in a community that takes into account both the overall abundance and the evenness (uniformity of abundance) of the groups present (in this case, Orders). This measure of biodiversity was chosen as it is the one most commonly used. The equation for the index is:

$$H = -\sum_{i=1}^{s} p_i \, ln p_i$$

H is the Shannon diversity index value, **s** is the total number of different groups in the community (richness), and $\mathbf{p_i}$ is the proportion representing the number of individuals belonging to one of the groups found divided by the total number of individuals found at the site. Average individual counts (calculated from the three sampling rounds at a site) were used instead of total counts across sampling rounds because each sampling round at the sites was considered a repeated sample.

Finally, two-sample unequal variance T-tests comparing non-averaged total count data from each round at each of the Invaded (Control) sites as a whole to the Successful Removal Sites as a whole were performed. This same comparison was performed between the Invaded (Control) sites and the Removed with Some Comeback sites. Each sampling round within a type of site was also compared to the sampling rounds from the other sites. For example, the total number of invertebrates found at all Invaded sites during the first round of sampling was compared with the total number of invertebrates found at all Successfully Removed sites during the first round of sampling. The purpose of these T-tests was to determine whether or not there was a significant difference in the abundance of the terrestrial invertebrates found at each site. In addition, T-tests were performed on selected Orders of terrestrial invertebrates (Coleoptera, Diplopoda, and Stylommatophora) to identify any differences between sites.

Two-sample unequal variance T-tests comparing the total dry weights of the terrestrial invertebrates found at each type of site were also performed in order to see if the biomass of the invertebrates was significantly different between the Invaded (Control) sites and the Successful Removal and Removed with Some Comeback sites. For the biomass T-tests, the total dry weight from each round at each of the Invaded (Control) sites was compared to the same type of data from the Successful Removal sites and then the Removed with Some Comeback sites. Each sampling round within a type of site was also compared to the sampling rounds from the other sites in another round of T-tests. Prior to analysis, 3 dry weights were removed from the dataset due to measurement error. These were Glen 14, Round 2 Hymenoptera; Glen 8, Round 1 (Isopoda); and Glen 5, Round 3 Orthoptera. Hutcheson T-tests were used to compare the Shannon Diversity Index values of all of the Invaded (Control) sites with all of the Successful Removal sites and then all of the Invaded (Control) sites with all of the Removed with Some Comeback sites.

Results

Across all sites and sampling rounds, 15 Orders of terrestrial invertebrates were identified. These Orders were: Coleoptera, Stylommatophora, Diplopoda, Isopoda, Pseudoscorpiones, Diptera, Hymenoptera, Lepidoptera, Haplotaxida, Acari, Araneae, Chilopoda, Tetromerocerata, Opiliones, and Orthoptera. Of these 15 Orders, Stylommatophora, Coleoptera, Diplopoda, and Diptera were the most common across sites. These Orders were present at all of the 10 sites in some amount. Tetromerocerata, Opiliones, and Pseudoscorpiones were the least common across all sites, each being present at only 1 site (John 1, Glen 5, and Glen 14, respectively; **Table 2**).

Table 2. Total individual counts by Order (combining all 3 sampling rounds) for each site.

	Invaded (Control)			Successfully Removed				Removed with Some Comeback		
Order	**Glen 4**	**Glen 6**	**Jaco 1**	**Glen 7**	**Glen 8**	**Glen 14**	**Glen 15**	**Glen 3**	**Glen 5**	**John 1**
Coleoptera	10	20	4	17	12	21	16	11	19	29
Stylommatophora	109	40	7	32	36	36	22	37	63	28
Diplopoda	148	123	7	54	66	55	78	42	231	41
Isopoda	0	2	1	0	2	1	0	0	12	5
Pseudoscorpiones	0	0	0	0	0	1	0	0	0	0
Diptera	6	4	2	4	21	4	4	2	5	7
Hymenoptera	2	4	0	2	6	5	7	1	2	0
Lepidoptera	0	1	0	4	0	1	1	1	2	1
Haplotaxida	0	2	9	3	5	12	1	3	2	0
Acari	0	4	0	1	0	1	0	0	0	1
Araneae	0	2	1	0	2	2	0	2	1	3
Chilopoda	0	3	6	2	3	0	0	0	1	4
Tetromerocerata	0	0	0	0	0	0	0	0	0	1
Opiliones	0	0	0	0	0	0	0	0	1	0
Orthoptera	0	0	0	0	0	0	0	0	2	1
Total	275	205	37	119	153	139	129	99	341	121

Of the 10 sites, Glen 5, a Removed with Some Comeback site, had the highest number of Orders present – 12 of the 15 being found at this site. Glen 4, an Invaded (Control) site, had the lowest number of Orders present – only 5 of the 15 were found at this site (**Table 2**).

Of the 10 sites, total biomass was highest at Glen 5, a Removed with Some Comeback site and Glen 4, an Invaded (Control) site. The lowest total biomass was found at Jaco 1, an Invaded (Control) site, the second lowest total biomass was found at John 1, Removal with Some Comeback site, and the third lowest total biomass was found at Glen 15, a Successfully Removed site. For many of the sites, the majority of the terrestrial invertebrate biomass came from 2 Orders – Stylommatophora and Diplopoda. Jaco 1 (Invaded), Glen 8 (Successfully Removed), Glen 14 (Successfully Removed), and Glen 3 (Removed with Some Comeback) all had relatively high Haplotaxida biomasses. Pseudoscorpiones and Tetromerocerata accounted for very low portions of the total biomass of the sites that they were found at (0.013 mg and 0.014 mg, respectively) (**Table 3**).

The diversity of the terrestrial invertebrates found at the individual sites does not show a clear trend regarding the effect of honeysuckle removal. Jaco 1 (Invaded) had the highest calculated Shannon Diversity value, the next highest was John 1 (Partial Removal), and the third highest was Glen 14 (Successful Removal). Glen 4 (Invaded) had the lowest Shannon Diversity value, Glen 5 (Partial Removal) was the next lowest, and Glen 15 (Successful Removal) was the third lowest (**Figure 4**).

The Invaded sites had a slightly higher average and greater spread of the number of individual terrestrial invertebrates found at the site when compared to the other site types as shown in **Figure 5** and **Figure 6**. This trend continued with biomass as shown in **Figure 7** and **Figure 8**.

Table 3. Total dry weight in milligrams (mg) by Order (combining all 3 sampling rounds) for each site; N/A denotes that after removal of erroneous data points, there was not enough data to give a total dry weight.

	Invaded (Control)			**Successfully Removed**				**Removed with Some Comeback**		
Order	**Glen 4**	**Glen 6**	**Jaco 1**	**Glen 7**	**Glen 8**	**Glen 14**	**Glen 15**	**Glen 3**	**Glen 5**	**John 1**
Coleoptera (Adult)	0.641	1.546	0.224	2.085	0.521	0.687	2.301	0.944	2.246	2.109
Coleoptera (Larvae)	0.617	0.27	0.243	0.346	0.547	0.159	0.587	0.96	0.246	0.111
Stylommatophora	21.855	15.336	2.352	5.925	7.685	5.981	2.315	11.32	15.772	8.938
Diplopoda	47.739	20.534	1.361	10.752	7.91	9.184	9.646	6.667	66.975	3.077
Isopoda	0	0.013	0.141	0	N/A	0.013	0	0	0.786	0.039
Pseudoscorpiones	0	0	0	0	0	0.013	0	0	0	0
Diptera (Adult)	0	0	0	0	0.042	0	0	0	0	0
Diptera (Larvae)	0.305	0.463	0.045	0.145	0.555	0.444	0.198	0.074	0.166	0.735
Hymenoptera (Adult)	0	0.052	0	0.112	0.071	0.016	0.035	0.026	0.128	0
Hymenoptera (Larvae)	0.154	0	0	0	0	0.101	0.18	0	0	0
Lepidoptera (Larvae)	0	1.407	0	1.654	0	0.381	1.95	0.035	0.73	1.698
Haplotaxida	0	0.016	10.664	3.751	7.918	18.939	3.135	11.764	1.394	0
Acari	0	0.019	0	0.021	0	0.021	0	0	0	0.011
Araneae	0	0.05	0.034	0	0.032	0.024	0	0.021	0.023	0.058
Chilopoda	0	0.277	0.125	0.091	0.132	0	0	0	0.023	0.133
Tetromerocerata	0	0	0	0	0	0	0	0	0	0.014
Opiliones	0	0	0	0	0	0	0	0	0.233	0
Orthoptera	0	0	0	0	0	0	0	0	N/A	0.024
Total	71.311	39.983	15.189	24.882	25.413	35.963	20.347	31.811	88.722	16.947

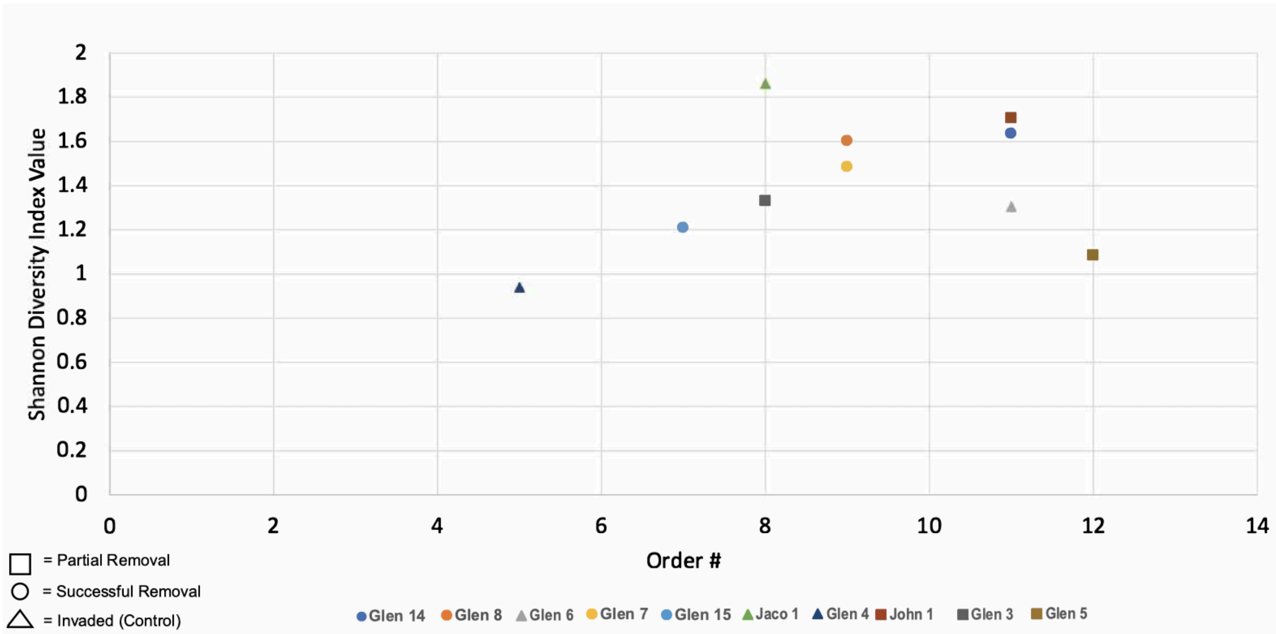

Figure 4. Calculated Shannon Diversity Index values for each site along with the number of Orders found at that site.

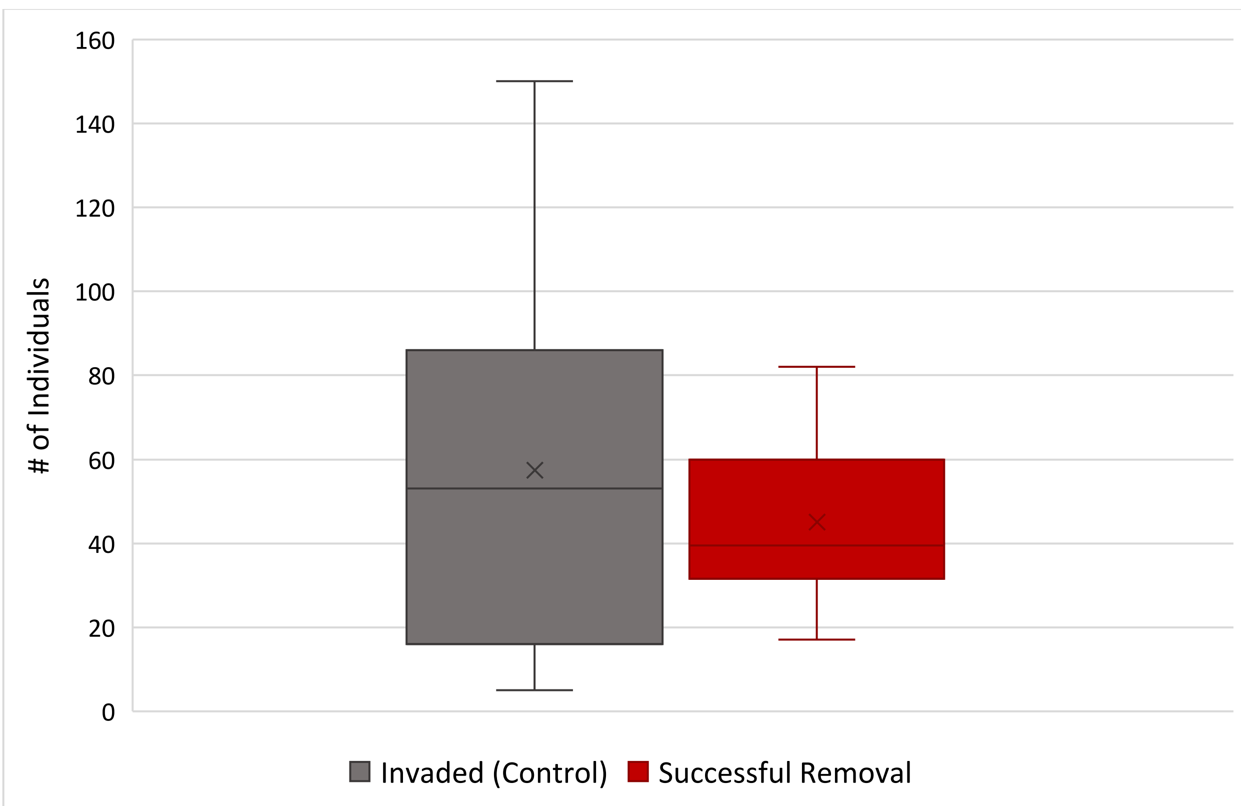

Figure 5. Total number of individuals found at the Invaded sites vs. the total number of individuals found at the Successful Removal sites

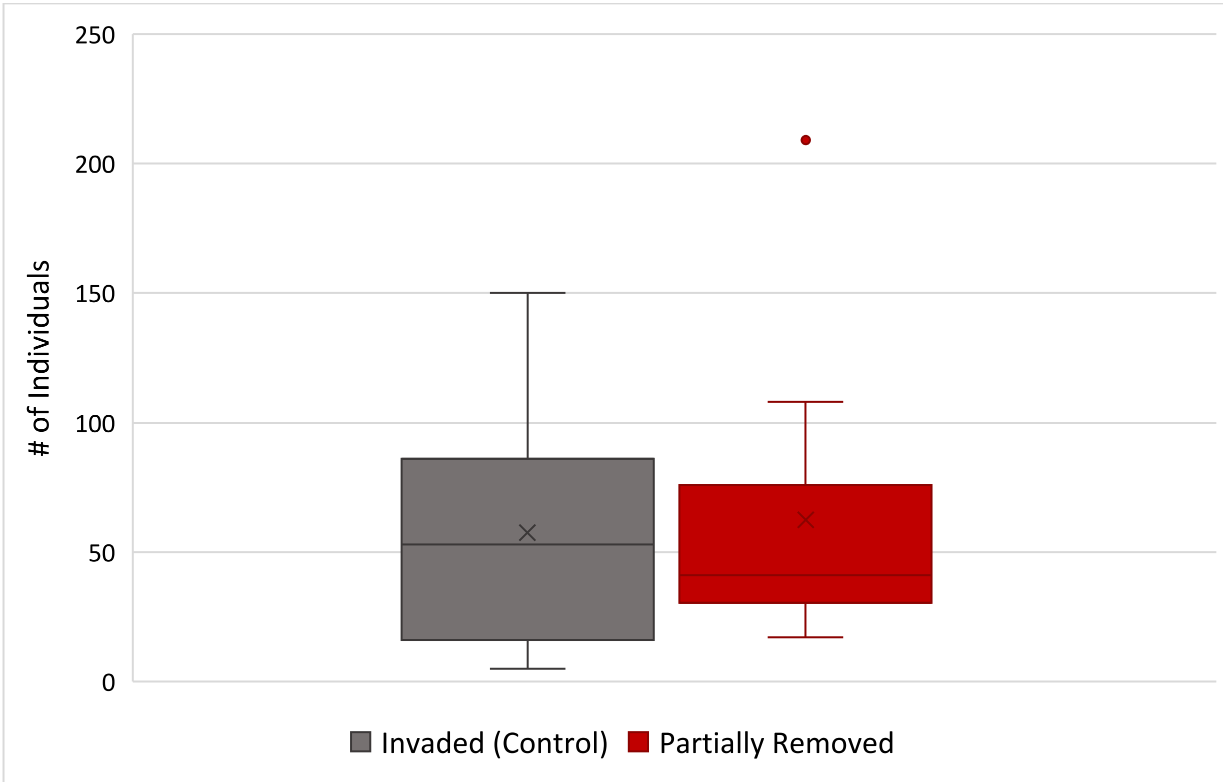

Figure 6. Total number of individuals found at the Invaded sites vs. the total number of individuals found at the Partially Removed sites

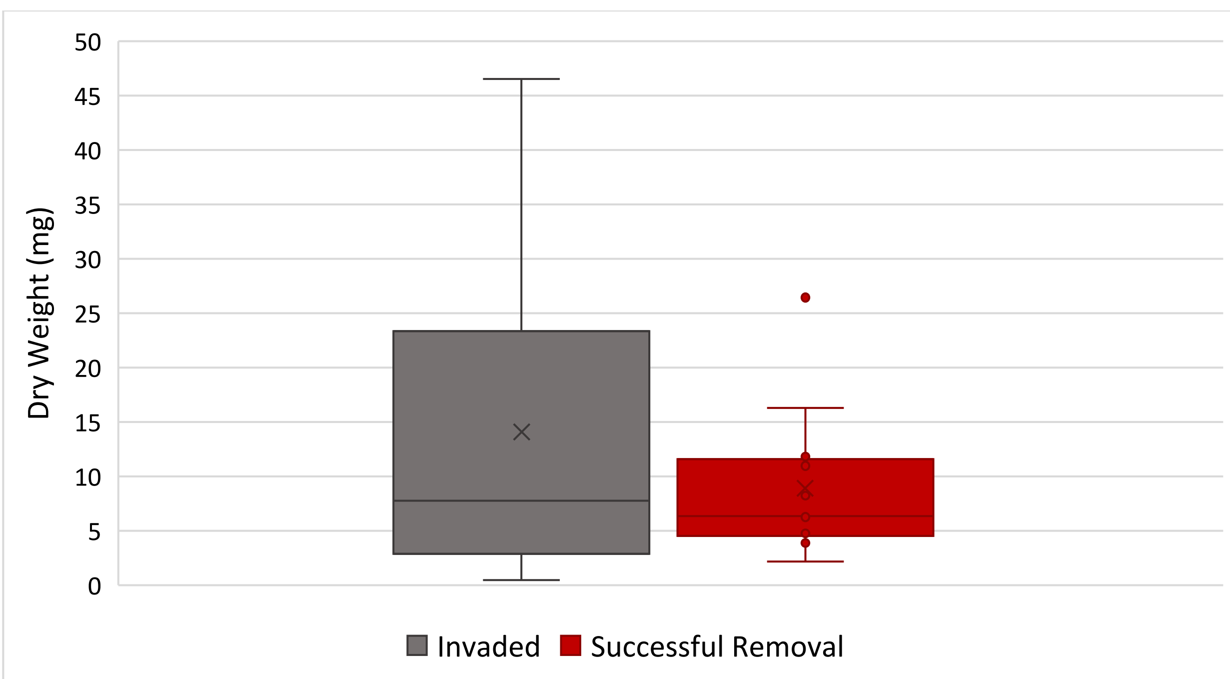

Figure 7. Total dry weight of the invertebrates found at the Invaded sites vs. the total dry weight of the invertebrates found at the Successful Removal sites

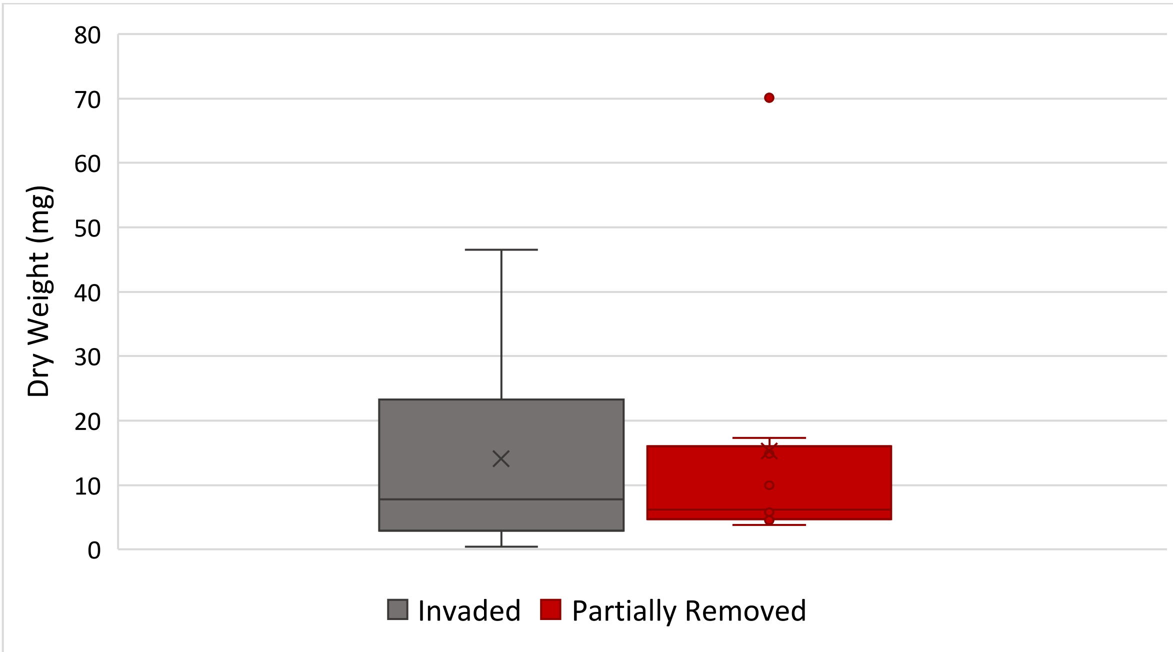

Figure 8. Total dry weight of the invertebrates found at the Invaded sites vs. the total dry weight of the invertebrates found at the Partially Removed sites

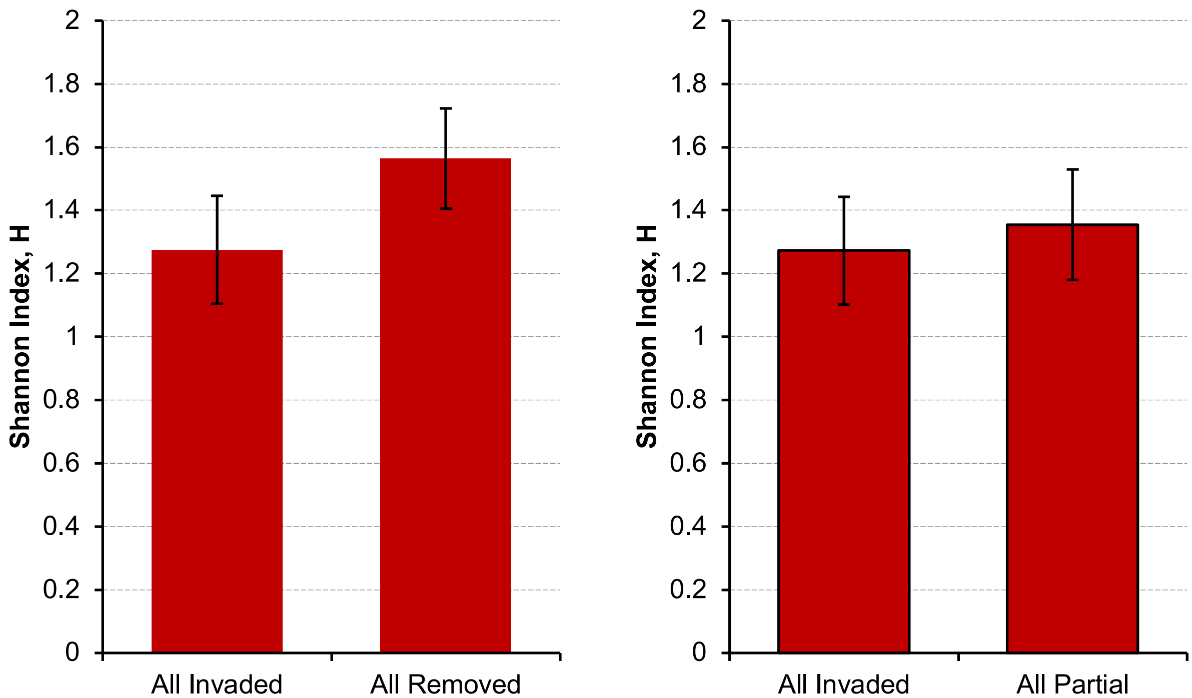

Figure 9. Results from the Hutcheson T-tests on Shannon Diversity Index between sites invaded of honeysuckle and site with some level of honeysuckle removal. The error bars indicate a 95% confidence interval

The Hutcheson T-tests showed that although the Shannon Diversity value for all of the Invaded sites was slightly lower than the value for all of the Removed and all of the Partial Removed sites, these differences were not statistically significant (**Figure 9**). There were no significant differences between the sites invaded of honeysuckle and those with some level of honeysuckle removal in all groups compared based on a 0.05 p-value. Table 3 gives a breakdown of select T-tests performed and the results of each.

Table 4. Results of selected T-tests

T-test Performed	Two-Tailed P-Value
Total Count for All Invaded vs. All Successful Removal Sites	0.470
Total Count for All Invaded vs. All Partially Removed Sites	0.851
Total Coleoptera Count for All Invaded vs. All Successful Removal Sites	0.347
Total Coleoptera Count for All Invaded vs. All Partially Removed Sites	0.218
Total Diplopoda Count for All Invaded vs. All Successful Removal Sites	0.404
Total Diplopoda Count for All Invaded vs. All Partially Removed Sites	0.831
Total Stylommatophora Count for All Invaded vs. All Successful Removal Sites	0.260
Total Stylommatophora Count for All Invaded vs. All Partially Removed Sites	0.657
Dry Weight for All Invaded vs. All Successful Removal Sites	0.362
Dry Weight for All Invaded vs. All Partially Removed Sites	0.890

Discussion

The data analysis did not find a significant difference in the abundance, biomass, or diversity of the invertebrates found at the different types of sites. There was also not a significant difference when the top three most abundant Orders (Coleoptera, Stylommatophora, & Diplopoda) were analyzed separately. The results of the research study suggest that removal of honeysuckle in riparian areas does not have an effect on the diversity, abundance, or biomass of the terrestrial invertebrates found at the site. The conclusion that overall abundance was not affected by honeysuckle would be in line with the findings of Christopher & Cameron (2012), who found that overall arthropod abundance was not significantly impacted by the presence of honeysuckle.

It is important to note that this study only identified the invertebrates to Order. Similar studies exploring the topic of invasive honeysuckle and its effects on arthropods and other terrestrial invertebrates, such as Loomis & Cameron (2013) and Masters et al. (2017), also only identified to the levels of taxonomic Order or Family. These studies did find some significant effects between the invertebrates in honeysuckle invaded and honeysuckle absent or removed plots, so it is likely that the lack of significance found in this study was not due to a lack of taxonomic specificity. It would, however, be interesting to repeat this study and identify the terrestrial invertebrates to the Species level. Throughout this project and the review of the existing literature, no previous studies doing so were found, and so this could shed new light on the effects of honeysuckle on these organisms.

It was interesting to find that there was no difference in the abundance of the top three Orders (Coleoptera, Stylommatophora, & Diplopoda) because the members of all three of these Orders are largely herbivorous. The study by McEwan and others (2008) found that the chemical compounds present in honeysuckle that deterred gypsy moths (a generalist herbivore) from eating them. This lack of significance may be explained by the ability of honeysuckle to interact with the environment in more ways than are readily apparent. As the study by Watling and others (2011) found, honeysuckle has the ability to create microclimates through temperature alterations due to growth density. This could have created a more favorable microclimate for these leaf litter-dwelling organisms and offset the possible effects of having an abundance of potentially unpalatable honeysuckle leaves. Future research may want to explore this further by including temperature measurements in addition to vegetation surveys and leaf litter sampling.

The study by Christopher & Cameron (2012) found that Acari were positively impacted by the presence of honeysuckle and that their abundance was higher in plots invaded by honeysuckle. As the raw count data in **Table 2** shows, the counts for Acari were highest at Glen 6, an Invaded (Control) site. However, there were not enough individuals in the sample for significance to be found in this. A future study may want to study Acari by themselves and include additional sites and sampling rounds in order to draw a more complete conclusion. As ticks are a member of the Acari Order, findings linking honeysuckle to ticks could have implications for reducing the spread of tick-borne diseases. **Table 2** also shows that Lepidoptera (all of which were in their larval stage), were overall most abundant at the Successful Removal sites. This would make sense due to the fact that honeysuckle leaves were found to be unpalatable by another member of the Lepidoptera Order (McEwan et al. 2008). The sample was quite small, so these numbers by themselves are not enough to make a significant conclusion, but future research may want to explore this in further detail.

Interestingly, the sites with the three highest individual Shannon Diversity Index values were Jaco 1, John 1, and Glen 14. These sites were all from different site type categories, showing that there was no clear trend in the diversity of the terrestrial invertebrates between the different types of sites. The site where the highest number of different invertebrate Orders were found was Glen 5, a site where removal had occurred, but then some honeysuckle had returned. These findings may reflect the differential impacts of honeysuckle on different species and its ability to benefit some while posing a null or detrimental effect to others.

This study solely focused on the impacts of honeysuckle removal on terrestrial invertebrates that were gathered at the ground level; it did not address flying insects. Previous studies have shown that flying insect species, including pollinators (McKinney & Goodell, 2010) and treehole mosquitoes (Conley et al., 2011), can be negatively affected by the presence of honeysuckle. Previous studies have also shown that butterflies may be positively impacted by the removal of invasive species that are ecologically similar to honeysuckle (Hanula & Horn, 2011). An important direction for future research could be addressing the effects of honeysuckle on flying insect species.

This study only focused on invasive honeysuckle as it was the dominant invasive at the sites that were studied. Other studies, however, have showed that invasive plants that co-occur can have combined effects on native species (Kuebbing et al., 2014). This indicates that the removal of co-occurring invasive species may be a valuable direction for future research.

The invertebrates in this study were collected during the months of May, June, and July. This time frame was chosen because this study was part of a larger research project which involved avian species, and thus the sampling period needed to be a time when the terrestrial invertebrates would be available as a food source for birds during their breeding season. Different terrestrial invertebrates emerge at different times, are most abundant during different seasons due to their specific needs, and have life-cycles of different lengths. This seasonality likely impacted the Orders and life stages found during the study. Sampling throughout the year may be able to provide a clearer picture of the invertebrates present at different times and the impact of honeysuckle on them.

Conclusion

This research study found that there was no significant difference in the abundance, biomass, nor diversity between the three different site types – invaded with no removal, successful removal, and removed with partial re-invasion – in riparian areas. This finding would imply that removal of honeysuckle does not make a difference for terrestrial invertebrate communities. These findings suggest that management strategies that focus on honeysuckle removal as a way to address the negative impacts of honeysuckle on native species can continue without too much concern for negative impacts to terrestrial invertebrate communities and possible trophic impacts that may occur as a result. This study serves to further expand our knowledge of honeysuckle and its impacts on the health of riparian ecosystems. It is important to note that this research study was specifically looking at the effects of honeysuckle in riparian areas, and so these findings may not hold for ecosystems where there are different environmental factors at play.

As these findings are not in agreement with some of the previous literature that is available, future research may want to further explore this area. Future research could identify the terrestrial invertebrates to Species to better understand the differential impacts of honeysuckle on invertebrate communities. A more specific level of taxonomic identification may be able to pick up on effects that were not apparent when identifying to the level of Order. Additional factors, such as temperature and moisture, that may create differential microclimates may warrant exploration. It may also be interesting to explore the specific type of honeysuckle removal on terrestrial invertebrate communities, as some methods may be more disruptive than others. Future studies may also wish to explore the same question, but in a different ecological setting that is not a riparian area.

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